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## Short communication

## Genetic diversity and inbreeding in the Greek Skyros horse

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**Abstract**

Inbreeding and heterozygosity levels were calculated in a population of 77 horses of the endangered Skyros breed that had been raised since 1988 at an experimental farm. Twenty one horses were inbred with average inbreeding coefficient of 0.11 ( $\pm 0.02$ ). Annual inbreeding change over the last 10 years was not significant ( $P > 0.05$ ). Animals were genotyped for 18 microsatellite markers. Average number of alleles was 4.11 ( $\pm 0.43$ ). Average theoretical and observed heterozygosity were 0.63 ( $\pm 0.06$ ) and 0.66 ( $\pm 0.06$ ), respectively. The probability of paternity exclusion with 1 and 2 parents unconfirmed was 0.9890 and 0.9999, respectively. Genetic diversity levels were reasonable and comparable to results from other breeds internationally.

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**Keywords:** Inbreeding; Diversity; Skyros horse

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**1. Introduction**

Inbreeding and heterozygosity characterize genetic diversity. Maintaining the latter is crucial for the evolution and improvement of animal species and can be a challenge for small populations. High inbreeding and low heterozygosity levels are often associated with reduced fitness, reproductive capacity and survival (Falconer and MacKay, 1996). Adverse effects of inbreeding on reproduction (Klemetsdal and Johnson, 1989; van Eldik et al., 2006) and survival (Curik et al., 2003) of horses have been documented.

Skyros is a small-sized horse originating in the Greek island of the same name. Average adult withers height is 1.09 m for males and 1.07 m for females. Purebred

Skyros horses are mainly bay (Fig. 1). Although these animals were previously used as drought horses, their value is increasingly recreational and cultural.

The breed is considered endangered. Currently less than 200 purebred Skyros can be found throughout the country. An unspecified number that is believed to be near 140 animals are raised on the island. As these horses roam freely the island pastures, they are often crossed with donkeys; the latter poses a severe challenge on the future of the breed. Phenotypically, the offspring of such crosses resemble considerably the purebred Skyros.

Over the past 18 years, a nucleus herd of purebred Skyros horses is being kept at an experimental farm of the Aristotle University of Thessaloniki in Northern Greece. Breeding is limited within the nucleus. At any given time, about 40 animals are raised at the farm. Because of the small number, it is important to monitor inbreeding and genetic diversity levels, and optimize the

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Fig. 1. Typical Skyros horses.

reproductive management of this population. To this date, the Skyros horse has not been studied genetically. Understanding the genetic background of the breed will increase its chances for survival.

The present study sets out to assess the genetic diversity, by quantifying levels of inbreeding and heterozygosity, in the University farm population of Skyros horses.

## 2. Materials and methods

### 2.1. Animal data

Data were from an experimental farm of the Aristotle University of Thessaloniki, Greece. A total of 77 purebred Skyros horses (40 females and 37 males) were considered. These animals had been raised on the farm since 1988. Mating was at random.

Using animal pedigrees, a genealogical tree was developed including all animals. Inbreeding coefficients for each animal were then calculated based on the genetic relationships among their parents. Inbreeding levels of common ancestors were also taken into account.

A total of 11 animals (7 females and 4 males) did not have any pedigree information. These were considered to be among

the population founding animals that were unrelated to each other.

### 2.2. Microsatellite marker genotyping

Animals were genotyped for 18 microsatellite markers. DNA was first extracted from blood samples and was then amplified with the use of polymerase chain reaction for polymorphism determination at the premises of Labogena in France. The individual marker nomenclature used was consistent with guidelines of the International Society of Animal Genetics (ISAG) and similar to Solis et al. (2005).

Allelic frequency was estimated using genotype counting. Based on allelic frequencies, theoretical heterozygosity levels were calculated for each marker with the following formula:

$$h_k = 1 - \sum p_i^2$$

where:

$h_k$	theoretical heterozygosity in marker $k$
$p_i$	frequency of allele $i$ in marker $k$
$\Sigma$	indicates that results are summed for each allele in marker $k$

Observed heterozygosity per marker was calculated as the proportion of the heterozygote individuals. The difference between expected and observed heterozygosity, expressed as a proportion of the former, was used to calculate heterozygote deficits per marker.

Molecular marker genotyping was also used to confirm parentage of the horses. The 9 markers recommended by the ISAG Equine Genetics Standing Committee for parentage testing were used for this matter. These were included in the 18 markers considered in the study.

## 3. Results and discussion

### 3.1. Inbreeding coefficients from pedigrees

Over a period of 18 years, 9 stallions and 20 mares had been randomly mated to produce the next generation of foals. Each male and female parent had an average of 7.3 and 3.3 progeny, respectively. In total, 21 horses (14 females and 7 males) were found to be inbred, representing 32% of the 66 non-founding animals. Average inbreeding was 0.11 ( $\pm 0.02$ ) for the 21 inbred horses and 0.03 ( $\pm 0.01$ ) for the 66 non-founding animals. Inbreeding coefficients ranged from 0.0234 to 0.25 and their frequency distribution is shown in Fig. 2. All but one of the inbred horses are still living today; the one dead had an inbreeding coefficient of 0.25. Differences between males and females were not significant ( $P > 0.05$ ). Ten animals had inbreeding coefficients

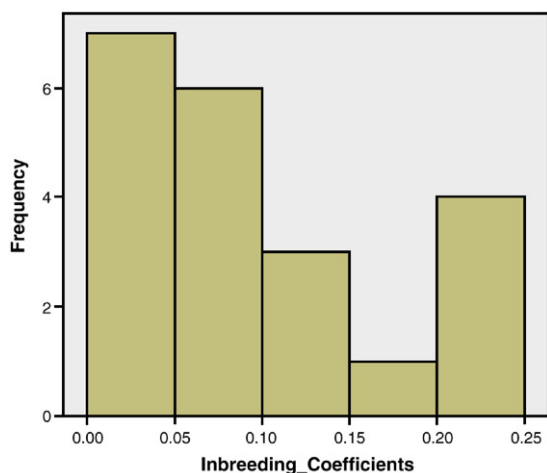


Fig. 2. Frequency of inbreeding coefficients (21 inbred horses).

greater than 0.0625, which, for practical purposes, may be considered as the maximum allowable for farm animal species. On three occasions, progeny had resulted from father–daughter matings, reaching the maximum inbreeding of 0.25. Looking at inbreeding levels by birth-year of the animals, no significant differences were detected. Over the past 10 years, the proportion of inbred animals increased by 0.9% ( $\pm 1.8\%$ ,  $P=0.65$ ) per year whereas the average annual inbreeding change was 0.002 ( $\pm 0.009$ ,  $P=0.81$ ).

The inbreeding profile of this population is similar to that reported by van Eldik et al. (2006) for a small population of Shetland ponies and within the range reported by Aberle et al. (2004) for six German horse breeds. Slightly higher inbreeding levels were found in a Central-Eastern European population of Lipizzan horses (Curik et al., 2003).

### 3.2. Genetic variability from genotyping

Table 1 summarizes results regarding estimates of genetic variability in the 18 microsatellite markers. Average number of alleles per locus across all markers was 4.11 ( $\pm 0.43$ ). Maximum number of alleles (8) was observed for marker ASB23 and minimum (2) on marker HTG6. Two markers (HTG4 and HMS1) were monomorphic. Amongst the 16 polymorphic markers, the most recurrent allele had an average frequency of 0.48 ( $\pm 0.04$ ) with a maximum value of 0.80 for marker HMS6. Solis et al. (2005) reported an average number of alleles in 12 microsatellite markers ranging between 5.75 and 8.08 for 4 horse breeds from the Basque country (Spain). They also reported an average of 4.33 alleles in thoroughbred horses, which is closer to our estimates.

Across the 16 polymorphic markers considered in the present study, theoretical heterozygosity levels ranged from 0.41 to 0.79 with an average of 0.63 ( $\pm 0.06$ ), whereas mean observed heterozygosity was 0.66

Table 1  
Genetic variability in 18 microsatellite markers of the Skyros horse

Marker	Number of alleles	Range of allele frequency	Heterozygosity		Heterozygote deficit
			Theoretical	Observed	
HTG6	2	0.46–0.54	0.4973	0.3902	0.2153
VHL20	3	0.23–0.44	0.6452	0.7073	–0.0964
HTG10	5	0.01–0.39	0.7249	0.6829	0.0579
HTG4	1	–	–	–	–
AHT5	5	0.06–0.35	0.7299	0.8293	–0.1361
AHT4	4	0.04–0.45	0.6565	0.7805	–0.1889
HMS3	4	0.11–0.65	0.5405	0.6098	–0.1282
HMS6	3	0.01–0.80	0.3186	0.3902	–0.2250
HMS7	7	0.05–0.33	0.7924	0.8537	–0.0773
HMS1	1	–	–	–	–
ASB2	4	0.18–0.32	0.7374	0.8537	–0.1577
ASB17	6	0.02–0.39	0.7213	0.7805	–0.0821
ASB23	8	0.01–0.43	0.7539	0.4167	0.4473
CA425	5	0.06–0.50	0.6675	0.8537	–0.2790
HTG3	3	0.06–0.74	0.4122	0.4500	–0.0917
HTG7	4	0.02–0.49	0.6279	0.7073	–0.1265
LEX3	4	0.03–0.49	0.6391	0.6750	–0.0562
LEX33	5	0.01–0.40	0.6673	0.6571	0.0153
Average	4.11		0.6332	0.6649	–0.0568
Standard error	0.43		0.0596	0.0668	0.0419

( $\pm 0.06$ ). This led to an overall heterozygote deficit of  $-0.06$  ( $\pm 0.04$ ). In 12 of 16 polymorphic markers (75%), heterozygote deficit was negative, meaning more heterozygotes were observed than expected from allelic frequencies. Solis et al. (2005) reported similar levels of theoretical and observed heterozygosity, ranging between 0.63 and 0.78 for five horse breeds (Pottoka and Jaca Navarra ponies, Euskal Herriko Mendiko Zaldia and Burguete medium-size horses, and thoroughbreds). Other heterozygosity estimates found in the literature include 0.67 ( $\pm 0.02$ ) for Lipizzan horses (Curik et al., 2003), 0.69 ( $\pm 0.03$ ) for 9 North American horse breeds (Colling and Kelly 1996), 0.65 ( $\pm 0.02$ ) for the Kladruber horse (Horin et al., 1998), and 0.64 ( $\pm 0.06$ ) for 4 Norwegian breeds (Bjørnstad et al., 2000). Despite its small size and isolation, the Skyros population studied here seems to have maintained reasonable levels of genetic diversity, at least by international standards.

The probability of paternity exclusion for these markers with 1 and 2 parents unconfirmed was 0.9890 and 0.9999, respectively, suggesting that these markers can be useful for parentage verification. Similar values were reported by Solis et al. (2005) for five horse breeds.

#### 4. Conclusions

Results from the present study suggest that, despite its small size, the Skyros horse population raised at an experimental farm of the Aristotle University of Thessaloniki has maintained reasonable levels of genetic diversity. It is now important to determine the relationship of genetic diversity parameters (inbreeding, heterozygosity) with reproductive performance and survival traits. This information will then be used to optimize the reproductive management and improve the breed's chances for survival.

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